Journal of Cancer Research and Clinical Oncology
https://doi.org/10.1007/s00432-021-03648-w

ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Application of circulating genetically abnormal cells in the diagnosis of early-stage lung cancer

Xiaochang Qiu[1] · Haoran Zhang[2] · Yongheng Zhao[3] · Jing Zhao[4] · Yunyan Wan[4] · Dezhi Li[4] · Zhouhong Yao[4] · Dianjie Lin[4]

Received: 2 February 2021 / Accepted: 19 April 2021

Abstract

Purpose Lung cancer is the leading cause of cancer-related death worldwide. The early detection of lung cancer is crucial for the diagnosis of this disease. Therefore, an effective and noninvasive method for the early diagnosis of lung cancer is urgently needed.

Methods To evaluate the diagnostic performance of circulating genetically abnormal cells (CACs) in early lung cancer, a total of 63 participants who completed CAC detection by Zhuhai SanMed Biotech Inc. and obtained pathological results from January to December 2020 were included in our study; 50 patients had lung cancer and 13 patients had benign lung disease. The levels of lung cancer-related markers in peripheral blood and the chest computed tomography (CT) imaging characteristics of these patients were collected before pathological acquisition.

Results The positive rate of CAC was 90.0% in the lung cancer group and 23.1% in the benign lung disease group, and the difference was statistically significant ($P < 0.01$). The area under the receiver operating characteristic (ROC) curve of CAC was 0.837, the sensitivity was 90%, and the specificity was 76.9%. The area under the ROC curve and sensitivity were both higher than those of the combined or single serum tumor marker test.

Conclusions This study preliminarily concludes that the CAC test, as a noninvasive test, has high sensitivity and specificity for the early diagnosis of lung cancer. This test is expected to help with the early detection of disease in lung cancer patients.

Keywords Circulating genetically abnormal cells · CAC · Lung cancer · Early diagnosis

Introduction

Lung cancer remains one of the malignant tumors that seriously threatens human safety. According to the latest global cancer data, lung cancer accounted for 11.1% (2.2 million) of new cancer cases worldwide in 2020, ranking second, and it ranked first in mortality (18%, 1.79 million). In 2020, 3 million people in China died from cancer, including 710,000 people who died of lung cancer, accounting for 23.8% of all cancer deaths (IARC 2020). These figures show that lung cancer seriously affects people's health. The prognoses of lung cancer patients with different clinical stages are significantly different (Woodard et al. 2016). It has been reported that due to the influence of different stages and regions, the 5-year survival rate of lung cancer patients ranges from 4 to 17%, and the primary reason for the poor prognosis of lung cancer is that most patients are in an advanced stage at the time of diagnosis, and many patients fail to show a treatment response (Hirsch et al. 2017). Therefore, the use of effective

✉ Zhouhong Yao
yaozhouhong@163.com

✉ Dianjie Lin
lindianjie@126.com

1 Shandong First Medical University Affiliated Provincial Hospital, Shandong First Medical University, Jinan 250021, Shandong Province, People's Republic of China

2 Department of Respiratory and Critical Care Medicine, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan 250021, Shandong Province, People's Republic of China

3 Shandong University, Cheeloo College of Medicine, Jinan 250021, Shandong Province, People's Republic of China

4 Shandong First Medical University Affiliated Provincial Hospital, Jinan 250021, Shandong Province, People's Republic of China

Published online: 24 April 2021

detection methods for the early diagnosis of lung cancer is expected to provide more options for subsequent treatment and is also the key to reducing the mortality rate.

Currently, imaging-based screening, tumor markers, and histopathological examination are the primary approaches for the diagnosis of lung cancer (Li et al. 2019). In 2006, the results of the International Early Lung Cancer Action Program published by The New England Journal of Medicine, including the United States, Europe, China and other countries, also highly affirmed the role of low-dose computed tomography (LDCT) in lung cancer screening. In addition, the Dutch-Belgian randomized lung cancer screening trial (NELSON) reached similar conclusions (van Iersel et al. 2007). The National Lung Screening Trial compared annual LDCT examinations with routine chest X-rays and found that after three rounds of screening, LDCT examination can reduce the mortality of lung cancer by 20% (Aberle et al. 2011). However, with the wide application of LDCT in lung cancer screening programs, the number of nodules detected in patients has also increased significantly, while the incidence of lung cancer has not increased correspondingly (Gould et al. 2015). This means that the increase in imaging and testing has produced more false positive results, failed to identify more cases of lung cancer, and may have caused patients to undergo unnecessary treatments due to overdiagnosis, which not only increases the economic burden of patients, but also causes anxiety, tension and other psychological problems (Gareen et al. 2014; Brodersen et al. 2020). Currently, liquid biopsies have emerged as a crucial tool in cancer management and are used to detect diagnostic biomarkers to identify patients with early-stage lung cancer or those at high risk of developing lung cancer. These biomarkers can often be detected in body fluids, such as saliva, urine and blood (Karachaliou et al. 2015; Siravegna et al. 2017). As a new noninvasive diagnostic technique, liquid biopsy has the advantages of easy and repeatable specimen acquisition compared with other screening methods for lung cancer and can be used for precision treatment, prognosis evaluation and efficacy monitoring of lung cancer (Poulet et al. 2019). A number of studies have reported the potential of liquid biopsy technology as a biomarker for the diagnosis of lung cancer, including cell-free DNA (Cohen et al. 2018), circulating tumor DNA (Blackhall et al. 2018) and exosomes (Shin et al. 2020).

One of the particularly important advances in the development of liquid biopsy was in 1869. Australian scholar Thomas Ashworth first observed tumor cells in the blood of patients with metastatic tumors and first proposed the concept of circulating tumor cells (CTCs) (Ferreira et al. 2016). CTCs are tumor cells that shed from the primary tumor and are released into the blood circulation. The migration of CTCs is an early event of cancer progression and has important application value in the early diagnosis of tumors (Yousefi et al. 2020). However, when CTC technology is used in the early diagnosis of lung cancer, the dependence on epithelial markers (EpCAM, etc.) should be overcome and CTCs should be distinguished from white blood cells in the blood at the same time (Mohan et al. 2017). In 2010, the American MD Anderson Cancer Center pathology professor Katz et al. (2010) found several common deletion or amplification genes in the peripheral blood mononuclear cells (PBMCs) of pulmonary squamous cell carcinoma and pulmonary adenocarcinoma; they developed a sensitive and quantitative antigen-independent four-color fluorescence in situ hybridization (FISH) technique to identify the genetic changes in chromosome 3 (3p22.1 and 3q29) and chromosome 10 (10q22.3 and CEP10) and named this type of PBMC with the same chromosomal locus mutation circulating genetically abnormal cell (CAC). However, there are still few studies on the application of CACs in the clinical diagnosis of lung cancer. Therefore, 63 patients with early lung cancer who completed the CAC test were included in this study. The results of this study showed that CAC and LDCT can be used to differentiate benign and malignant pulmonary nodules to improve the diagnostic accuracy and reduce unnecessary invasive tissue examinations.

Materials and methods

Study design and patients

A retrospective analysis was performed on 63 patients who were treated in Shandong, China between January and December 2020 and completed the CAC test by Zhuhai SanMed Biotech Inc. These patients were divided into two groups according to their postoperative histopathological diagnosis. In the lung cancer group, there were 27 males and 23 females, aged from 31 to 74 years, including 46 patients with lung adenocarcinoma, 2 patients with lung squamous cell carcinoma, and 2 patients with small cell carcinoma (SCLC). The clinical stages were TIS (5 patients), IA (37 patients), IB (7 patients), and IIB (1 patient). There were six males and seven females in the benign lung disease group, aged 34–78 years. There were no statistically significant differences in sex or age between the different groups.

The subjects included in this study all met the following criteria: (1) all patients were older than 18 years and from Shandong Province; (2) all patients completed the CAC test and obtained histopathological results after the CAC results; (3) all patients were in the early stage of lung cancer (stage TIS ~ IIB); (4) all patients had no history of malignancy and had not received any antitumor treatment before enrollment; and (5) all blood samples and computed tomography (CT) scans were obtained just prior to surgery. In addition, the measurement of tumor markers

was not required for enrollment in this study. Due to the retrospective nature of this study, we waived the requirement of informed consent, and the study was approved by the committee of Shandong First Medical University and explained the relevant matters in detail to the participants.

The diagnosis of benign disease was determined by imaging and histopathology. The staging criteria used were those from the TNM staging system of the American Joint Committee on Cancer (https://www.cancer.org/treatment/understanding-your-diagnosis/staging). Tumor pathology was classified according to the World Health Organization (WHO) classification standard of lung tumors (2015 edition) (Travis et al. 2015).

Methods

CAC detection

The CAC tests of all patients in our study used MDA TEST technology (a test originating from the MD Anderson Cancer Center in the United States centered on detecting CACs in the blood), which was performed by Zhuhai SanMed Biotech Inc. The CAC testing process was as follows. (1) Blood collection and fixation: fresh venous blood (8–10 ml) was collected with an EDTA vacuum anticoagulant tube, and matched cell preservation solution was added within 2 h, including solution A containing phosphatase inhibitor and protease inhibitor and solution B containing formaldehyde. The sample was gently inverted ten times and was stored and transported for up to 4 days at room temperature. (2) Target cell enrichment and purification: PBMCs were enriched by automatic enrichment or Ficoll density gradient centrifugation. (3) Glass slide preparation and hybridization: cell slides were prepared, and a fixation solution was added. After protease digestion, multicolor FISH was performed using a mononuclear cell chromosome abnormity detection kit. Image scanning and interpretation: the BioView automated abnormal cell scanning analyzer can automatically complete scanning, imaging and analysis in 30 min to observe the chromosome changes in the target locus. The CAC interpretation criteria were as follows: CAC count ≥ 3 indicates a positive test; CAC count < 3 indicates a negative test. Positive results indicate a higher malignant risk of pulmonary nodules, which should be evaluated in combination with imaging examinations, the clinical characteristics of patients and other tests. The patients were treated with nonsurgical biopsy, aggressive surgery, or close follow-up (3 months). For negative results, it is recommended to comprehensively evaluate the clinical information of imaging examinations and other tests and follow-up patients according to the existing diagnostic and treatment standards.

CT and tumor biomarkers

CT scanning and serum tumor marker determination were completed in normal hospitals in Shandong Province. By analyzing the CT data, the maximum diameter of lung nodules (specifically referring to the pathological subjects in this paper) in the lung cancer group was 4–30 mm, with the median and interquartile range being 14 mm and 9–18 mm, respectively. In the benign pulmonary disease group, the maximum diameter was 7–30 mm, and the median and interquartile range were 10 mm and 10–18 mm, respectively. The differences between the two groups was significant ($P < 0.001$), which indicates that the diameters of the nodules in the lung cancer group were generally higher than those in the benign lung disease group. According to the results of serum tumor marker determination, 65.7 pg/ml, 10 ng/ml, 6.0 ng/ml, 1.5 ng/ml, 16.3 ng/ml and 35 U/ml were considered as the normal upper limits of ProGRP, CEA, CYFRA21-1, SCC, NSE and CA125, respectively, and there were 3, 1, 2, 0, 9 and 1 people over the upper limit.

Statistical analysis

All statistical analyses were performed using SPSS (version 26, SPSS Inc., Chicago, IL). The receiver operating characteristic (ROC) curve was drawn by SPSS with sensitivity as the ordinate and 1-specificity as the abscissa to evaluate the diagnostic effect of CAC and other methods in detecting carcinoma of the lungs. For the continuous variables, we used the Mann–Whitney U test. For categorical variables, we used the χ^2 test for analysis. All P values were two-sided, and we considered $P < 0.05$ to indicate statistical significance.

Results

Basic characteristics of the patients

There were 63 patients in this study, including 50 (79.4%) patients with lung cancer and 13 (20.6%) patients with benign lung lesions. The patients were aged 31–78 years, including 33 males (52.4%) and 30 females (47.6%). The Kolmogorov–Smirnov test confirmed that age, nodule size, course of disease and tumor markers in the two groups had skewed distribution data, so the median and interquartile range were selected to represent the results. The results showed that the time from the discovery of nodules to the operation in the lung cancer group was longer than that in the benign lung lesion group, and the difference was statistically significant ($P < 0.001$) (the data of two patients were missing, $n = 61$). This might be related to the time spent in the follow-up dynamic CT examination of patients

with lung cancer before the operation. Nodules with diameters ≤ 10 mm were found in the lung cancer and benign lesion groups, accounting for 38% and 53.8%, respectively. The majority of lung cancer patients had mixed ground-glass nodules, accounting for 44%, while in benign lung disease patients, solid nodules were the most common, accounting for 61.5%. In this study, 49 patients completed CEA, CYFRA21-1 and NSE measurement, 48 patients completed ProGRP measurement, 47 patients completed SCC measurement, and 44 patients completed CA125 measurement. None of the patients had a history of occupational dust exposure. The positive rate of CACs in lung cancer patients was significantly higher than that in the control group ($P < 0.001$). The basic characteristics of the two groups are shown in Table 1.

Table 1 Demographic characteristics of lung cancer group and benign lung diseases group

Characteristics	*N* (proportion)	Lung cancer group ($n = 50$)	Benign lung diseases group ($n = 13$)	*P*
Gender, n(%)				0.614
Male	33 (52.4%)	27 (54%)	6 (46.2%)	
Female	30 (47.6%)	23 (46%)	7 (53.8%)	
Age (year)				0.052
Median, (interquartile range)	57 (50,62)	58 (50,62)	57 (49,61)	
Smoking history, n (%)				1.000
Yes[a]	20 (31.7%)	16 (32%)	4 (30.8%)	
No	43 (68.3%)	34 (68%)	9 (69.2%)	
Family history of cancer, n (%)				0.298
Yes[b]	14 (22.2%)	13 (26%)	1 (7.7%)	
No	49 (77.8%)	37 (74%)	12 (92.3%)	
Course of the disease [c](month)				
Median, (interquartile range)	2 (1,10)	2 (1,13)	1 (1,8)	< 0.001
Tumor biomarkers Median, (interquartile range)				
ProGRP(pg/ml)	32.45 (23.88,39.84)	31.41 (23.94,40.63)	27.35 (22.35,40.56)	0.049
CEA(ng/ml)	1.64 (1.14,2.63)	1.64 (1.08,2.59)	2.06 (1.12,3.66)	0.143
CYFRA21-1(ng/ml)	2.24 (1.45,3.20)	2.22 (1.53,3.05)	2.20 (1.45,3.33)	< 0.001
SCC(ng/ml)	0.60 (0.50,0.90)	0.70 (0.50,0.90)	0.60 (0.50,1.10)	0.170
NSE(ng/ml)	14.82 (12.88,15.67)	14.87 (12.83,15.98)	13.55 (12.51,15.35)	< 0.001
CA125(U/ml)	7.80 (6.00,9.83)	7.44 (6.00,9.96)	8.70 (5.68,9.58)	0.062
Nodule size[d] (n/%) (mm)				0.546
≤ 10	26 (41.3%)	19 (38%)	7 (53.8%)	
10–20	22 (34.9%)	18 (36%)	4 (30.8%)	
> 20	15 (23.8%)	13 (26%)	2 (15.4%)	
Nodule type (n/%)				0.006
Solid nodule	17 (27.0%)	9 (18%)	8 (61.5%)	
Mixed ground-glass nodule	26 (41.3%)	22 (44%)	4 (30.8%)	
Pure ground-glass nodule	20 (31.7%)	19 (38%)	1 (7.7%)	
Special signs of nodules (n/%)				
Lobulation	20 (31.7%)	17 (34%)	3 (23.1%)	0.675
Spiculation	34 (54.0%)	27 (54%)	7 (53.8%)	0.992
Pleural indentation	16 (25.4%)	13 (26%)	3 (23.1)	1.000
Vessel convergence sign	35 (55.6%)	28 (56%)	7 (53.8)	0.889
Vacuolar sign	7 (11.1%)	6 (12%)	1 (7.7%)	1.000
CAC ≥ 3 (n/%)	48 (76.2%)	45 (90%)	3 (23.1%)	< 0.001

[a]Include current smokers or patients with a history of smoking

[b]Parent or sibling with a malignant tumor

[C]Time from the first discovery of the nodule to the completion of the operation

[d]Maximum diameter of the nodule

Relationship between CAC positivity and clinical characteristics

We aimed to clarify whether there was a difference in positive CAC expression according to the basic characteristics of the 63 subjects. The data are summarized in Table 2. The results showed that there were no significant differences between CAC positivity and the participants' demographics including age ($P=0.977$), sex ($P=0.612$), family history ($P=1.000$), smoking history ($P=1.000$), and tumor nodule size ($P=0.770$). Among the different pathological types, lung adenocarcinoma was the most common, with 46 cases (92%), and the positive rate of CAC was 89.1% in these patients. It should be noted that the CAC positive rate was 100% for both squamous cell carcinoma and SCLC patients, possibly due to the small number of patients included (two patients each). The CAC positive rate of stage TIS (5 patients) and IIb (1 patient) patients was 100%, and there was no statistically significant difference from the 89.2% positive rate of stage IA patients (37 patients). We found that the CAC positive rate of patients with solid nodules (95%) was significantly higher than that of patients with pure ground-glass nodules (52.9%), with a statistically significant difference ($P=0.005$); however, there was no significant difference in CAC positive rate between mixed ground-glass nodules (76.9%) and nodules with other two densities. At the same time, for continuous variables such as the course of disease and blood tumor biomarkers, we performed the Man Whitney *U* test and found that the course of disease of patients with CAC-positive test results was significantly longer than that of patients with CAC-negative test results ($P=0.049$). Moreover, CYFRA21-1 ($P=0.007$), SCC ($P=0.001$), and NSE ($P<0.001$) were significantly different between the two groups.

Table 2 Relationship between CAC positivity and clinical characteristics

Characteristics	CAC ≥ 3 (*n*/%)	CAC < 3 (*n*/%)	*P*
Gender			0.612
Male	26 (78.8%)	7 (21.2%)	
Female	22 (73.3%)	8 (26.7%)	
Age, years			0.977
≥ 60	19 (76%)	6 (24%)	
< 60	29 (76.3%)	9 (23.7%)	
Smoking history			1.000
Yes[a]	15 (75%)	5 (25%)	
No	33 (76.7%)	10 (23.3%)	
Family history of cancer			1.000
Yes[b]	10 (76.9%)	3 (23.1%)	
No	38 (76%)	12 (24%)	
Pathological types			1.000
Adenocarcinoma[c]	41 (89.1%)	5 (10.9%)	
Squamous cell carcinoma	2 (100%)	0 (0%)	
SCLC	2 (100%)	0 (0%)	
TNM stage			1.000
Tis	5 (100%)	0 (0%)	
IA	33 (89.2%)	4 (10.8%)	
IB	6 (85.7%)	1 (14.3%)	
IIB	1 (100%)	0 (0%)	
Nodule size[d] (mm)			0.770
≤ 10	21 (80.8%)	5 (19.2%)	
10–20	16 (72.7%)	6 (27.3%)	
> 20	11 (73.3%)	4 (26.7%)	
Nodule type			0.008
Solid nodule	9 (52.9%)	8 (47.1%)	
Mixed ground-glass nodule	20 (76.9%)	6 (23.1%)	
Pure ground-glass nodule	19 (95%)	1 (5%)	
Special signs of nodules			
Lobulation	16 (80%)	4 (20%)	0.868
Spiculation	28 (82.4%)	6 (17.6%)	0.214
Pleural indentation	10 (62.3%)	6 (37.6%)	0.137
Vessel convergence sign	24 (68.6%)	11 (31.4%)	0.197
Vacuolar sign	4 (57.1%)	3 (42.9%)	0.433

SCLC small cell lung cancer

[a]Include current smokers or patients with a history of smoking

[b]Parent or sibling with a malignant tumor

[C]Include 5 cases of adenocarcinoma in situ, 8 cases of minimally invasive adenocarcinoma and 33 cases of invasive adenocarcinoma

[d]Maximum diameter of the nodule

Diagnostic efficacy of the CAC test

Previous results indicated that the CAC positive rate of solid nodules was significantly higher than that of ground-glass nodules, and the course of disease of CAC-positive participants was significantly longer than that of CAC-negative participants. Therefore, we used the Spearman test, which showed that the correlation coefficient between the CAC positive rate and course of disease was 0.045, and the correlation coefficient between CAC positive rate and nodular density was −0.430, both of which were not significantly correlated. Through ROC curve analysis (Figs. 1, 2, 3, 4, 5, 6, 7, 8), we found that the area under the curve (AUC) of CAC counts was 0.837 (95% confidence interval (CI), 0.810–0.864, $P<0.001$). In this result, using ≥ 2.5 CACs was the positive standard, and we obtained a sensitivity of 90.8% and a specificity of 83.9%. This result is also close to the CAC positive limit. The ROC curve of CACs was higher than that of the combined or single serum tumor marker test (Figs. 1, 2, 3, 4, 5, 6, 7, 8; Table 3). It should be noted that the ROC curve of the CAC test was strange in shape, and the region of 0.1–0.4 on the X axis showed a decreasing trend, which may be caused by the small sample size. The data in Table 1 also showed that the positive rate of CAC in

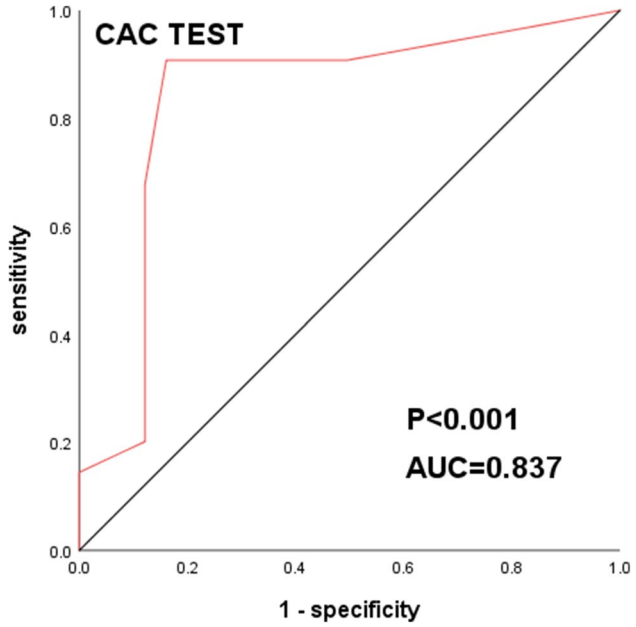

Fig. 1 ROC curve analysis of lung cancer detected by CAC test

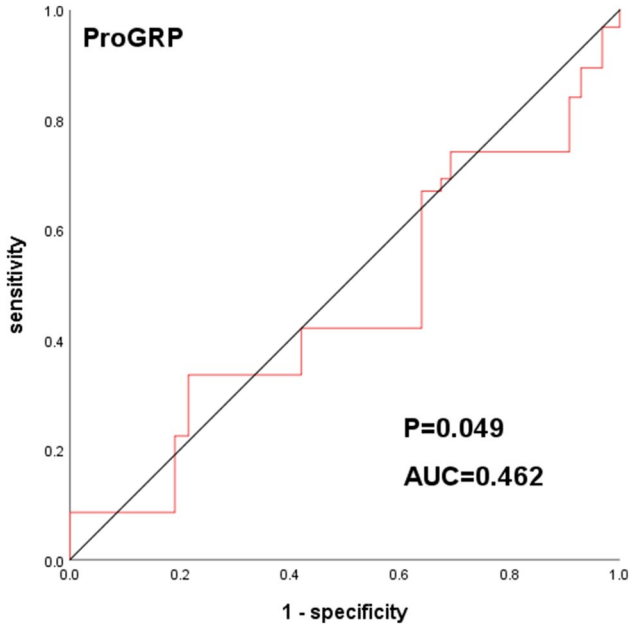

Fig. 3 ROC curve analysis of lung cancer detected by ProGRP

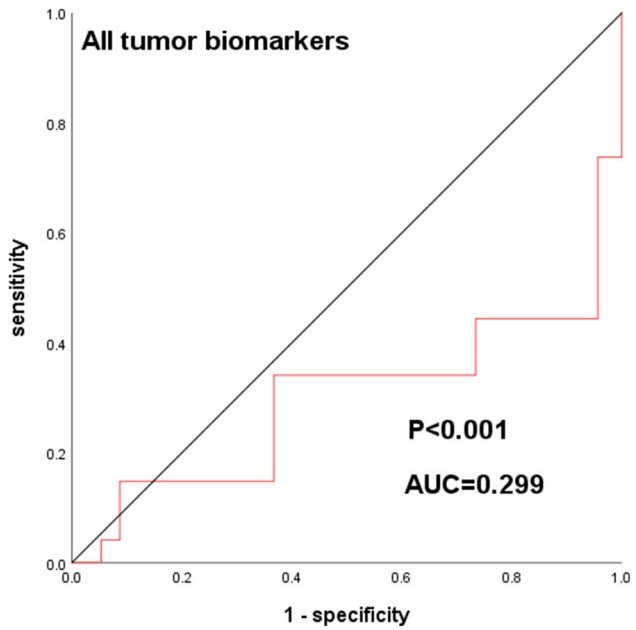

Fig. 2 ROC curve analysis of lung cancer detected by all tumor biomarkers

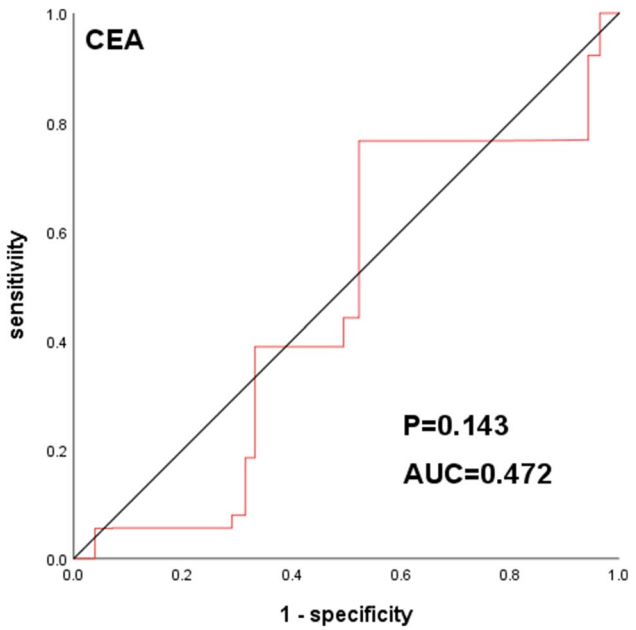

Fig. 4 ROC curve analysis of lung cancer detected by CEA

all participants was 76.2%, and the positive rate in the lung cancer group (90%) was significantly higher than that in the benign lung lesion group (23.1%, $P < 0.01$). Therefore, we can conclude that the CAC test may have good application value in the diagnosis of early lung cancer.

At the same time, we also compared the diagnostic efficacy of the CAC test with that of the blood tumor markers and specific signs of nodules in chest CT (Table 3), including the sensitivity, specificity, accuracy and AUC (the AUC has not yet been evaluated because the specific signs of nodules are classified variables). The results showed that the sensitivity (90%) and accuracy (87.3%) of the CAC test

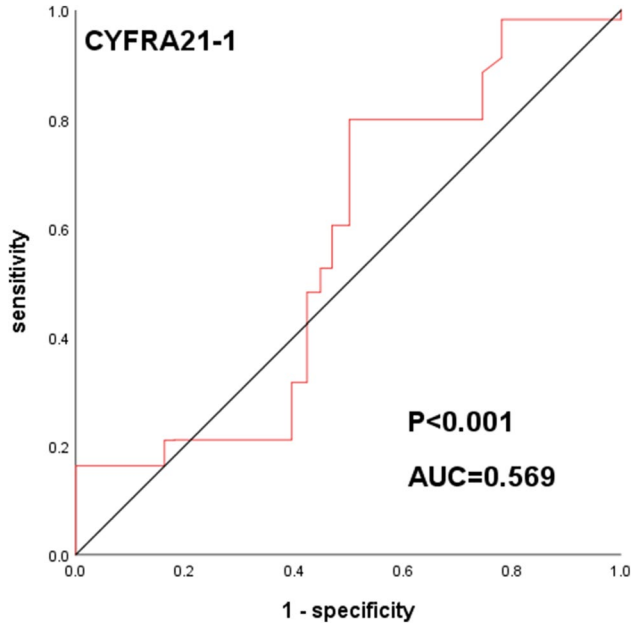

Fig. 5 ROC curve analysis of lung cancer detected by CYFRA21-1

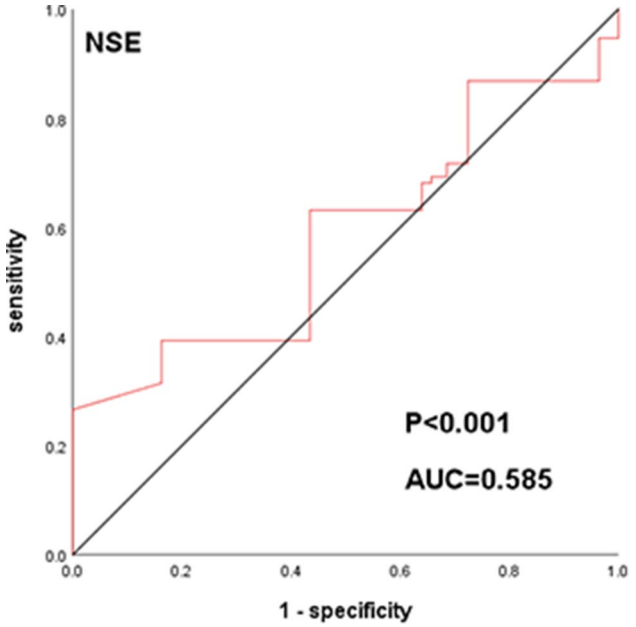

Fig. 7 ROC curve analysis of lung cancer detected by NSE

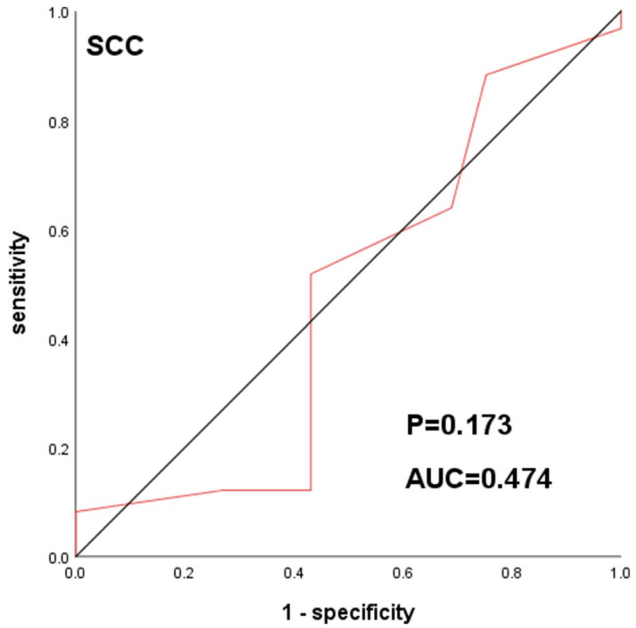

Fig. 6 ROC curve analysis of lung cancer detected by SCC

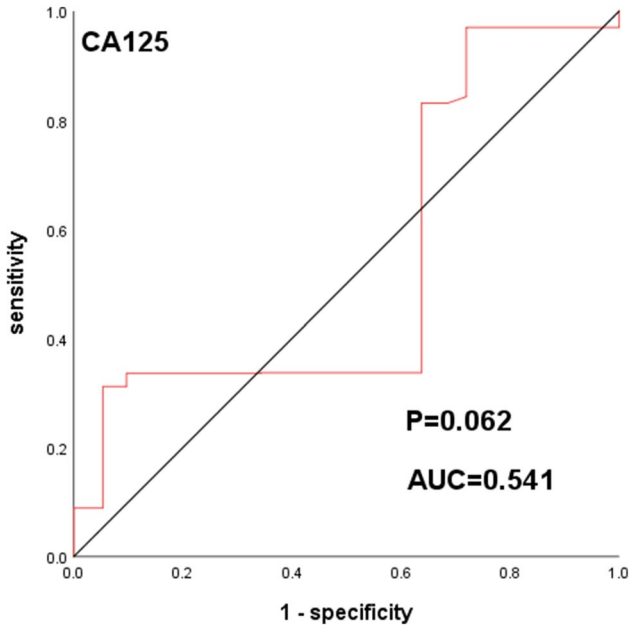

Fig. 8 ROC curve analysis of lung cancer detected by CA125

for the diagnosis of lung cancer were higher than those of other detection methods, including the combination of tumor biomarkers (37.1% sensitivity and 46.5% accuracy). In our study, the sensitivity, specificity, accuracy and AUC of NSE were 23.7%, 100%, 40.8% and 0.585 (95% CI 0.552–0.618), which were all higher than those of other single blood tumor markers. It should be noted that the *P* values of CEA, SCC, and CA125 analyzed in the ROC curve analysis were all above 0.05, and the results were not considered to be meaningful. This finding may be related to the small amount of overall data (1 CEA, 1 CA125, and 0 SCC positive cases), with certain statistical errors. Among all the nodule signs reported on chest CT, the vessel convergence sign had high sensitivity (56%) and accuracy (54%), while the vacuolar

Table 3 Comparison in different diagnostic methods

Diagnostic methods	N^b	Sensitivity	Specificity	Accuracy	AUC (95% CI)
CAC test	63	90.0%	76.9%	87.3%	0.837 (0.810–0.864)
ProGRP	48	8.1%	100%	29.2%	0.462 (0.426–0.498)
CEA	49	0	90.9%	20.4%	0.472 (0.430–0.513)
CYFRA21-1	49	5.3%	100%	26.5%	0.569 (0.527–0.611)
SCC	47	0	100%	23.4%	0.474 (0.431–0.516)
NSE	49	23.7%	100%	40.8%	0.585 (0.552–0.618)
CA125	44	2.8%	100%	20.5%	0.541 (0.495–0.586)
All tumor biomarkers[a]	43	37.1%	87.5%	46.5%	0.299 (0.268–0.329)
Lobulation	63	34%	76.9%	42.9%	–
Speculation	63	54%	46.2%	52.4%	–
pleural indentation	63	28%	76.9%	38.1%	–
vessel convergence sign	63	56%	46.2%	54%	–
vacuolar sign	63	12%	92.3%	28.6%	–

Sensitivity, specificity and accuracy all depend on the positive criteria of various detection methods to obtain data, AUC obtains results based on the continuous values of each detection method

AUC area under curve

[a]All tumor biomarkers, include ProGRP + CEA + CYFRA21-1 + SCC + NSE + CA125; positive means that any one of them is positive

[b]Refer to the number of people who have completed each test

sign had the highest specificity (92.3%) for the diagnosis of lung cancer.

Discussion

It is well known that lung cancer is a multifactorial and highly aggressive cancer and is the common cause of cancer-related death worldwide (Torre et al. 2015). The etiology of lung cancer is still unknown, but cancer and chronic respiratory disease are both linked to tobacco use (Cao and Chen 2019). Smoking is recognized as the leading risk factor for lung cancer, but other risk factors, such as air pollution, biomass burning, and occupational exposure (asbestos), also play an important role in the development of lung cancer (Bade and Dela Cruz 2020). Lung cancer is generally divided into non-small cell lung cancer (NSCLC, including lung adenocarcinoma, lung squamous cell carcinoma and large cell lung cancer) and small cell lung cancer according to pathological type, accounting for 85% and 15% of all lung cancers, respectively. Significant survival differences between patients with different T and M stages of lung cancer have been reported, including differences in survival among patients with single-site or multisite metastases involving the brain or other sites (Nicholson et al. 2016; Carter et al. 2018). Therefore, to effectively prevent the occurrence of death from lung cancer, in addition to avoiding or reducing the exposure to risk factors as much as possible, appropriate early diagnosis is particularly important.

LDCT screening significantly reduces lung cancer mortality in high-risk populations by detecting early-stage disease, according to the results of a large randomized controlled trial published in the August 2011 issue of the New England Journal of Medicine (Aberle et al. 2011). Based on this finding, the American Cancer Society (ACS), American College of Chest Physicians (ACCP) and other lung cancer screening guidelines have adopted the same inclusion criteria for the target population: annual LDCT screening for people aged 55–74 years, who have a smoking index of ≥ 30 pack years, are actively smoking or have quit smoking within the past 15 years and have no other life-limiting comorbidity (Detterbeck et al. 2013; Wender et al. 2013; Smith et al. 2014). However, with the improvement of people's awareness of health examinations and the popularization of LDCT applications and due to the high sensitivity and lack of specificity of CT, the detection rate of clinical pulmonary nodules is increasing, which may lead to an increase in invasive treatment and has a potential harmful risk (Tanoue et al. 2015). Currently, pulmonary nodules are defined as focal, quasi-round, dense solid or subsolid pulmonary opacity ≤ 3 cm in diameter; they can be a solitary pulmonary nodule or multiple pulmonary nodules and can be benign or malignant, among which malignant nodules are lung cancer (Chinese Medical Journal 2018). The diagnosis of pulmonary nodules is mainly evaluated by clinical information, imaging techniques, and surgical and nonsurgical biopsies. For low-risk patients, LDCT scans should be repeated in long-term follow-up to compare the external structure (nodule size, shape, edge,

etc.) and internal characteristics (nodule density, structure, etc.) to help distinguish benign and malignant pulmonary nodules (Chinese Medical Journal 2018). The correct differentiation of benign and malignant pulmonary nodules is helpful for the early surgical treatment of malignant nodules and the improvement of patient prognosis.

Dr. Ruth L. Katz of the MD Anderson Cancer Center, as the technical inventor of the MDA Test, developed a four-color FISH technique to identify cytogenetic changes and proposed the concept of CACs as distinct cells from CTCs (Katz et al. 2010). This advance suggests that there are genetic abnormalities in patients with non-small cell lung carcinoma that are similar to those in the primary tumor and are strongly associated with the presence and early development of cancer. Because the test is antigen-independent, it may show more CACs if it is not restricted by the antigen detection of epithelial cell differentiation. In Katz et al. (2020) used a novel antigen-independent method of four color FISH to detect CTCs with abnormal copy number mononuclear cells in the peripheral blood of patients with lung cancer ($n = 107$) and non-lung cancer ($n = 100$), and obtained results with an accuracy of 94.2%, a sensitivity of 89%, and a specificity of 100%. Moreover, Liu et al. (2020) analyzed 261 lung cancer patients and 78 healthy participants in 2020 and concluded that the number of CACs in early-stage NSCLC patients was significantly higher than that in healthy subjects, and the sensitivity of CAC detection in the identification of NSCLC was 67.2% (higher than that of tumor markers), with a specificity of 80.8%. These studies all suggest that CACs may be an effective, specific biomarker for the diagnosis of tumors, with high potential in being accurate.

In this study, we counted the number of peripheral blood CACs in 63 patients in the 2 groups. After determining that there were no significant differences in sex, age and smoking history between the two groups of patients, the results showed that the CAC positive rate (90%) was significantly higher in the lung cancer group than in the benign lung disease group (23.1%). This difference was statistically significant, which is in agreement with previous research results suggesting that CACs may be a potential biomarker for the early diagnosis of lung cancer. Since it has been previously reported that different nodular features are helpful for the diagnosis of lung cancer (McWilliams et al. 2013; Sihong et al. 2017), we added indicators such as density, size and special CT signs of nodules in this study. Through further analysis, we found that the CAC positive rate of patients with solid nodules (95%) was significantly higher than that of patients with pure ground glass nodules (52.9%), and there was a statistically significant difference in the course of disease between patients with CAC-positive nodules and those with CAC-negative nodules. Therefore, we conducted correlation analysis and found that the correlation coefficient between the CAC positive rate and course of disease was 0.045, and that between the CAC positive rate and the density of nodules was −0.430, both of which were not significant correlations. After that, we also compared the diagnostic efficacy of CAC and tumor markers and the special appearance of nodules on CT. The AUC of the CAC count was 0.837 ($P < 0.001$), which was higher than that of the combined or single tumor marker test. The sensitivity (90%) and accuracy (87.3%) of CAC in the diagnosis of lung cancer were higher than those of other detection methods, including tumor biomarker combination detection (37.1% sensitivity and 46.5% accuracy) and special signs of nodules [vessel convergence sign had the highest sensitivity (56%) and accuracy (54%) for the diagnosis of lung cancer, and vacuolar signs in the diagnosis of lung cancer had the highest specificity (92.3%)]. This indicates that CAC detection has high sensitivity and specificity in the early-stage diagnosis of lung cancer, which is basically consistent with the results of the research by the experts mentioned previously.

As a result, we can conclude that noninvasive CAC detection may have a higher detection rate than tumor markers and chest CT in the diagnosis of early-stage lung cancer. Although tumor markers have certain reference significance for the diagnosis of lung cancer, traditional serum tumor markers are mostly related to the pathological types of lung cancer; for example, CYFRA21-1 (Yu et al. 2017) is often used as a serum tumor marker of NSCLC, and NSE (Yang et al. 2018) is often used as a serum tumor marker for small cell lung cancer. However, when we analyzed the relationship between positive CAC expression and the pathological types of lung cancer, it was found that the positive expression of CAC in lung cancer was not affected by the pathological types of lung cancer patients. Therefore, CAC detection has more advantages when applied in the diagnosis of lung cancer. Of course, if CAC detection is used for the early diagnosis of lung cancer, one must consider that its price is almost ten times that of LDCT, which increases the economic burden of patients. This study shows that CAC has some false positive or false negative results, but its clinical application value is undeniable. The high positive rate and accuracy of the CAC test make it helpful in the identification of early-stage lung cancer, especially uncertain pulmonary nodules, and can even make an earlier diagnosis than CT scans. Although the CAC test cannot replace CT scans, it can help patients select further treatment plans and is an effective auxiliary test. However, due to the limited number of samples included in this study, such as only one patient being positive for CEA, one patient being positive for CA125 and zero patients being positive for SCC, there may be some statistical errors, and further verification of the accuracy of the results of this study is needed in a large sample population. At present, led by Professor Chunxue Bai, the Chinese Alliance for Lung Cancer Prevention and

Treatment and SanMed Biotech jointly launched the BaiDX & MDA TEST for the auxiliary diagnosis of malignant pulmonary nodules in a national multicenter clinical study. A total of 13 regional key tertiary class A hospitals are participating in the study, and the study plans to include more than 1000 patients with pulmonary nodules. MDA TEST technology was combined with artificial intelligence imaging in a prospective validation study on benign and malignant pulmonary nodules. We hope that there will be a large sample of MDA test data in China in the near future.

Availability of data and material The data and materials are true and valid.

Declarations

Conflict of interest There is no conflict of interest involved in this study.

Ethics approval The study was approved by the ethics committee of Shandong First Medical University.

References

Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, Fagerstrom RM, Gareen IF, Gatsonis C, Marcus PM, Sicks JD (2011) Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 365:395–409. https://doi.org/10.1056/NEJMoa1102873

Bade BC, Dela Cruz CS (2020) Lung cancer 2020: epidemiology, etiology, and prevention. Clin Chest Med 41:1–24. https://doi.org/10.1016/j.ccm.2019.10.001

Blackhall F, Frese KK, Simpson K, Kilgour E, Brady G, Dive C (2018) Will liquid biopsies improve outcomes for patients with small-cell lung cancer? Lancet Oncol 19:e470–e481. https://doi.org/10.1016/s1470-2045(18)30455-8

Brodersen J, Voss T, Martiny F, Siersma V, Barratt A, Heleno B (2020) Overdiagnosis of lung cancer with low-dose computed tomography screening: meta-analysis of the randomised clinical trials. Breathe (Sheff) 16:200013. https://doi.org/10.1183/20734735.0013-2020

Cao M, Chen W (2019) Epidemiology of lung cancer in China. Thorac Cancer 10:3–7. https://doi.org/10.1111/1759-7714.12916

Carter BW, Lichtenberger JP 3rd, Benveniste MK, de Groot PM, Wu CC, Erasmus JJ, Truong MT (2018) Revisions to the TNM staging of lung cancer: rationale, significance, and clinical application. Radiographics 38:374–391. https://doi.org/10.1148/rg.2018170081

Cohen JD, Li L, Wang Y et al (2018) Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 359:926–930. https://doi.org/10.1126/science.aar3247

Detterbeck FC, Mazzone PJ, Naidich DP, Bach PB (2013) Screening for lung cancer: diagnosis and management of lung cancer, 3rd ed: American college of chest physicians evidence-based clinical practice guidelines. Chest 143:e78S-e92S. https://doi.org/10.1378/chest.12-2350

Ferreira MM, Ramani VC, Jeffrey SS (2016) Circulating tumor cell technologies. Mol Oncol 10:374–394. https://doi.org/10.1016/j.molonc.2016.01.007

Gareen IF, Duan F, Greco EM, Snyder BS, Boiselle PM, Park ER, Fryback D, Gatsonis C (2014) Impact of lung cancer screening results on participant health-related quality of life and state anxiety in the National lung screening trial. Cancer 120:3401–3409. https://doi.org/10.1002/cncr.28833

Gould MK, Tang T, Liu IL, Lee J, Zheng C, Danforth KN, Kosco AE, Di Fiore JL, Suh DE (2015) Recent trends in the identification of incidental pulmonary nodules. Am J Respir Crit Care Med 192:1208–1214. https://doi.org/10.1164/rccm.201505-0990OC

Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ, Jr., Wu YL, Paz-Ares L (2017) Lung cancer: current therapies and new targeted treatments. Lancet 389:299–311. https://doi.org/10.1016/s0140-6736(16)30958-8. https://www.cancer.org/treatment/understanding-your-diagnosis/staging

IARC (2020) Latest global cancer data: cancer burden rises to 19.3 million new cases and 10.0 million cancer deaths in 2020. https://www.iarc.who.int/fr/news-events/latest-global-cancer-data-cancer-burden-rises-to-19-3-million-new-cases-and-10-0-million-cancer-deaths-in-2020/

Karachaliou N, Mayo-de-Las-Casas C, Molina-Vila MA, Rosell R (2015) Real-time liquid biopsies become a reality in cancer treatment. Ann Transl Med 3:36. https://doi.org/10.3978/j.issn.2305-5839.2015.01.16

Katz RL, He W, Khanna A et al (2010) Genetically abnormal circulating cells in lung cancer patients: an antigen-independent fluorescence in situ hybridization-based case-control study. Clin Cancer Res 16:3976–3987. https://doi.org/10.1158/1078-0432.ccr-09-3358

Katz RL, Zaidi TM, Pujara D, Shanbhag ND, Truong D, Patil S, Mehran RJ, El-Zein RA, Shete SS, Kuban JD (2020) Identification of circulating tumor cells using 4-color fluorescence in situ hybridization: validation of a noninvasive aid for ruling out lung cancer in patients with low-dose computed tomography-detected lung nodules. Cancer Cytopathol 128:553–562. https://doi.org/10.1002/cncy.22278

Li Y, Tian X, Gao L, Jiang X, Fu R, Zhang T, Ren T, Hu P, Wu Y, Zhao P, Yang D (2019) Clinical significance of circulating tumor cells and tumor markers in the diagnosis of lung cancer. Cancer Med 8:3782–3792. https://doi.org/10.1002/cam4.2286

Liu WR, Zhang B, Chen C et al (2020) Detection of circulating genetically abnormal cells in peripheral blood for early diagnosis of non-small cell lung cancer. Thorac Cancer 11:3234–3242. https://doi.org/10.1111/1759-7714.13654

Lung Cancer Research Group of Respiratory Society of Chinese Medical Association and Expert Group of China Lung Cancer Prevention and Control Alliance, Chinese experts consensus on the diagnosis and treatment of pulmonary nodules (2018) Chinese J Tuberculosis Respir Dis 41(10)

McWilliams A, Tammemagi MC, Mayo JR et al (2013) Probability of cancer in pulmonary nodules detected on first screening CT. N Engl J Med 369:910–919. https://doi.org/10.1056/NEJMoa1214726

Mohan S, Chemi F, Brady G (2017) Challenges and unanswered questions for the next decade of circulating tumour cell research in lung cancer. Transl Lung Cancer Res 6:454–472. https://doi.org/10.21037/tlcr.2017.06.04

Nicholson AG, Chansky K, Crowley J, Beyruti R, Kubota K, Turrisi A, Eberhardt WE, van Meerbeeck J, Rami-Porta R (2016) The International association for the study of lung cancer lung cancer staging project: proposals for the revision of the clinical and pathologic staging of small cell lung cancer in the forthcoming eighth edition of the TNM classification for lung cancer. J Thorac Oncol 11:300–311

Poulet G, Massias J, Taly V (2019) Liquid biopsy: general concepts. Acta Cytol 63:449–455. https://doi.org/10.1159/000499337

Shin H, Oh S, Hong S et al (2020) Early-stage lung cancer diagnosis by deep learning-based spectroscopic analysis of circulating exosomes. ACS Nano 14:5435–5444. https://doi.org/10.1021/acsnano.9b09119

Sihong C, Jing Q, Xing J, Baiying L, Tianfu W, Dong N, Jie-Zhi C (2017) Automatic scoring of multiple semantic attributes with multi-task feature leverage: a study on pulmonary nodules in CT images. IEEE Trans Med Imaging 36:802–814. https://doi.org/10.1109/tmi.2016.2629462

Siravegna G, Marsoni S, Siena S, Bardelli A (2017) Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol 14:531–548. https://doi.org/10.1038/nrclinonc.2017.14

Smith RA, Manassaram-Baptiste D, Brooks D, Cokkinides V, Doroshenk M, Saslow D, Wender RC, Brawley OW (2014) Cancer screening in the United States, 2014: a review of current American cancer society guidelines and current issues in cancer screening. CA Cancer J Clin 64:30–51. https://doi.org/10.3322/caac.21212

Tanoue LT, Tanner NT, Gould MK, Silvestri GA (2015) Lung cancer screening. Am J Respir Crit Care Med 191:19–33. https://doi.org/10.1164/rccm.201410-1777CI

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. CA Cancer J Clin 65:87–108. https://doi.org/10.3322/caac.21262

Travis WD, Brambilla E, Nicholson AG et al (2015) The 2015 world health organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol 10:1243–1260. https://doi.org/10.1097/jto.0000000000000630

van Iersel CA, de Koning HJ, Draisma G, Mali WP, Scholten ET, Nackaerts K, Prokop M, Habbema JD, Oudkerk M, van Klaveren RJ (2007) Risk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON). Int J Cancer 120:868–874. https://doi.org/10.1002/ijc.22134

Wender R, Fontham ET, Barrera E Jr et al (2013) American cancer society lung cancer screening guidelines. CA Cancer J Clin 63:107–117. https://doi.org/10.3322/caac.21172

Woodard GA, Jones KD, Jablons DM (2016) Lung cancer staging and prognosis. Cancer Treat Res 170:47–75. https://doi.org/10.1007/978-3-319-40389-2_3

Yang Q, Zhang P, Wu R, Lu K, Zhou H (2018) Identifying the best marker combination in CEA, CA125, CY211, NSE, and SCC for lung cancer screening by combining ROC curve and logistic regression analyses: is it feasible? Dis Markers 2018:2082840. https://doi.org/10.1155/2018/2082840

Yousefi M, Ghaffari P, Nosrati R, Dehghani S, Salmaninejad A, Abarghan YJ, Ghaffari SH (2020) Prognostic and therapeutic significance of circulating tumor cells in patients with lung cancer. Cell Oncol (Dordr) 43:31–49. https://doi.org/10.1007/s13402-019-00470-y

Yu Z, Zhang G, Yang M, Zhang S, Zhao B, Shen G, Chai Y (2017) Systematic review of CYFRA 21–1 as a prognostic indicator and its predictive correlation with clinicopathological features in non-small cell lung cancer: a meta-analysis. Oncotarget 8:4043–4050. https://doi.org/10.18632/oncotarget.14022